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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

A step-forward in the characterization and potential applications of solid and liquid oxygen transfer vectors

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Abstract Silicone oil 20 and 200 cSt, a perfluorocarbon (FC40TM), heptamethylnonane, Kraton, Elvax, and Desmopan were evaluated for their ability to enhance oxygen transfer in stirred tank and airlift reactors (STR and ALR, respectively). None of the vectors tested was either toxic or biodegradable and they exhibited a moderate affinity for oxygen (gas/vector partitioning coefficients $K_{g/v} = C_g \cdot C_v^{-1}$ ranging from 3 to 5.1). FC40 was highly volatile, while KratonTM and ElvaxTM exhibited a low thermal stability, which constitutes a serious handicap for their implementation in fermentations. Silicone oil 20 cSt and Desmopan supported the highest oxygen transfer rates under abiotic conditions in both STR and ALR designs, with enhancement factors of up to 90% and 250%, respectively, compared to control tests (deprived of vector). The fact that these vectors showed the highest $K_{g/v}$ proved that, besides the classical selection criteria, the in situ hydrodynamic behavior (which affects K_La) must be considered for vector selection. The use of silicone oil 20 cSt and Desmopan in glucose-supplemented *Saccharomyces cerevisiae* fermentations resulted in a two- and threefold increase in biomass productions, respectively. The better performance of Desmopan in terms of biomass growth enhancement, together with the absence of the operational problems inherent to the use of liquid vectors (such as intensive foaming, high cost, and difficult solvent recovery), make solid vectors a promising and cost-effective alternative in the future developments of two-phase partitioning bioreactors.

Keywords Oxygen transfer rate · *Saccharomyces cerevisiae* · Transfer vector · Vector selection · Two-phase partitioning bioreactors

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Introduction

Oxygen transfer from the gas to the aqueous phase constitutes a critical issue in industrial fermentations, often being the limiting step due to the low solubility of oxygen in water (Amaral et al. 2008; Dumont et al. 2006; Elibol and Mavituna 1999; Gomes et al. 2007). Enhancement in oxygen transfer rates has traditionally involved either the increase in stirring or aeration rates or the introduction of oxygen-enriched air streams, which significantly increases production costs (Garcia-Ochoa and Gomez 2009). Therefore, the development of low-cost methods capable of enhancing oxygen transfer is crucial for increasing the cost-effectiveness of industrial biotechnology.

The addition of a water-immiscible phase, also called transfer vector, represents an efficient alternative to help overcome the mass transfer limitations of poorly water-soluble compounds such as oxygen, hexane, and α-pinene (Cesario et al. 1997; Dumont and Delmas 2003; Littlejohns and Daugulis 2007; Muñoz et al. 2006; Muñoz et al. 2008). The presence of a non-aqueous phase (of solid or liquid nature) enhances gaseous substrate mass transfer because of the higher affinity that these hydrophobic substrates have for the vectors, increasing the overall concentration gradient for mass transfer. Moreover, an additional vector/water interfacial area for direct substrate uptake by microorganisms can be established (Deziel et al. 1999).

Several authors have demonstrated that alkanes, perfluorocarbons, vegetable oils, or silicone oils can significantly improve oxygen transfer in several bioreactor configurations (Clarke and Correia 2008; Elibol 2001; Galindo et al. 2000; Ho et al. 1990; Jia et al. 1996; Quijano et al. 2009). For instance, Nielsen et al. (2003) estimated an increase of 58% in the oxygen transfer capacity of a stirred tank reactor by using 27% of *n*-hexadecane under abiotic conditions. Likewise, Cesario et al. (1997) increased the oxygen transfer by 120% using 10% of the perfluorocarbon FC40. Solid polymers such as silicone rubber, Hytrel, Kraton, and Desmopan have recently been shown to enhance the transfer of oxygen and other poorly water-soluble compounds (Littlejohns and Daugulis 2007; Rehmann et al. 2007).

Traditionally, vector selection criteria have involved immiscibility, a high affinity for oxygen, no inhibition of microbial activity, non-biodegradability, non-volatility, and low cost (Bruce and Daugulis 1991; van Groenestijn and Lake 1999). These criteria, despite constituting a necessary condition for their use in two-phase partitioning bioreactors (TPPBs), are not sufficient to guarantee an enhanced oxygen transfer. Classical selection criteria must be therefore revised in order to account for the bioreactor design selected and the particular process requirements. Additionally, although numerous works on TPPBs have been published, most of them are made on a case-by-case basis and relatively little weight has been given to systematic vectors comparison.

This work carried out a systematic evaluation of the compliance of the most commonly used liquid and solid vectors (in both environmental and biotechnological fields) with the classical selection criteria. Silicone oil (20 and 200 cSt), a perfluorocarbon (FC40), heptamethylnonane (HMN), Kraton, Elvax, and Desmopan were selected as non-aqueous organic phases representative of liquid and solid vectors. The ability of these vectors to enhance oxygen transfer to the aqueous phase, as a key selection criterion, was evaluated in terms of the overall oxygen transfer rate in two reactor designs, a stirred tank reactor (STR) and an airlift reactor (ALR). In addition, the best performing liquid and solid vectors (in terms of oxygen transfer enhancement capacity under abiotic conditions and compliance with classical selection criteria) were further tested in glucose-supplemented *Saccharomyces cerevisiae* cultures in a STR, to evaluate their effect in a model biotechnological process (Baker's yeast production).

Materials and methods

Chemicals

The liquid vectors tested were: silicone oil (poly(dimethylsiloxane) with kinematic viscosities of 20 and 200 cSt), 2,2,4,4,6,8,8-heptamethylnonane, and the perfluorocarbon FC40TM. The solid vectors tested were: KratonTM G1657 (3–4 mm beads of styrene–ethylene/butadiene tribloc copolymer), DesmopanTM DP9370A (3×3 mm cylinders of polyurethane of poly(oxytetramethylene)glycol and methyldiisocyanate), and ElvaxTM 360 (3–4 mm beads of poly(ethylene-co-vinyl acetate)). Silicone oils and HMN were purchased from Sigma-Aldrich. The FC40, Kraton, Desmopan, and Elvax were kindly supplied by 3M, Kraton Polymers, Bayer, and Dupont, respectively.

Inoculum

S. cerevisiae (DSMZ 2155) was grown in a mineral salt medium (MSM), prepared according to Gombert et al. (2001) and enriched with glucose at 10 g L^{-1}. Sterile cultures of *S. cerevisiae* were incubated for 16 h in aerated (1.3×10^{-5} m^3 s^{-1}) 2-L glass flasks at 30°C.

Vector toxicity and biodegradability

Solvent toxicity was assessed in 120-mL glass flasks containing 2 mL of vector, 2 mL of *S. cerevisiae* inoculum, and 20 mL of sterile MSM enriched with glucose, yeast extract, and peptone at a final concentration of 1, 0.02, and 0.02 g L^{-1}, respectively. The flasks were closed with butyl septa, sealed with aluminum caps, and incubated for 90 h in an orbital shaker at 300 rpm and 25°C. Control flasks without a vector were also carried out. The headspace composition of the flasks (O_2/CO_2) was periodically analyzed by GC-TCD. The vector was considered toxic if oxygen depletion in the flasks was significantly lower ($p<0.05$) than that in the control tests. The tests were carried out in duplicate.

Biodegradability tests were conducted over a period of 30 days (in order to allow for microbial acclimation; OECD 1993) in a similar manner that the toxicity tests described above but in the absence of glucose, yeast extract, and peptone. Control tests without a vector were also prepared in order to account for bacterial endogenous respiration. Additional control flasks enriched with 1 g L^{-1} of glucose were also carried out in order to evaluate the culture viability. The vector was considered biodegradable if CO_2 production in the flasks was significantly higher ($p<0.05$) than that in the control tests. The tests were carried out in duplicate.

Solvent volatility

Poly(methyl methacrylate) aerated tubes (0.032 m inner diameter, 1 m height) were used to assess the potential volatility of the liquid vectors (silicone oil 20 cSt, silicone

oil 200 cSt, HMN, and FC40). The columns, containing 200 mL of vector, were aerated from the bottom using a porous sparger at 3.3×10^{-6} m^3 s^{-1}. The tests were maintained at room temperature (25°C) for 30 days. Vector volatilization was estimated by direct measurement of vector volume decrease.

Polymer thermal stability

The resistance of polymers to sterilization via autoclaving was performed according to Morrish and Daugulis (2008): 5 g of each polymer was added to 120-mL glass flasks containing either distilled water or MSM. The flasks were autoclaved at 121°C for 25 min and polymer thermal stability was evaluated by visual observation of polymer melting.

Gas/vector oxygen partitioning coefficient

The oxygen partitioning coefficient ($K_{g/v}$) in the liquid vectors was determined by measuring the oxygen concentration in the vector at equilibrium (C_v^*) with air and pure oxygen atmospheres. Glass flasks of 120 mL, containing 10 mL of a Na_2SO_3 solution (0.06 M), were deoxygenated by N_2 sparging. The flasks were then supplied with 1 mL of catalyst solution (to reach a final concentration of $CoSO_4$ of 2.5×10^{-4} M), closed with butyl septa and sealed with aluminum caps. Five milliliters of vector, previously saturated by the sparging of air (first series of experiments) or pure oxygen (second series) for 24 h, were injected into the flasks. The test flasks were incubated for 24 h under magnetic agitation (300 rpm) at 25°C in order to ensure the complete transfer of oxygen from the vector to the reactive sulfite solution. Flasks without a vector were prepared to serve as controls.

Likewise, Kraton, Desmopan, and Elvax were saturated by continuously supplying air (first series of experiments) or pure oxygen (second series) to plastic cartridges containing 2 mL of the solid vector for 24 h at a flow rate of 3.3 mL s^{-1}. Oxygen uptake by the plastic cartridges, although present, was not relevant to the determination of the oxygen partitioning coefficients since all solid polymers tested were saturated after 24 h of exposure to the different oxygen atmospheres (data not shown). The maximum oxygen concentration in the solid polymer was determined as described above. Flasks without a vector were prepared to serve as controls. $K_{g/v}$ was estimated by Eq. 1, where C_g represents the oxygen concentration in air or pure oxygen atmospheres:

$$K_{g/v} = \frac{C_g}{C_v^*} \quad (1)$$

Vector/water ethanol partition coefficient

The vector/water partitioning coefficient for ethanol ($K_{v/w} = C_v \cdot C_w^{-1}$) was experimentally determined in gas-tight 120-mL glass bottles supplied with 2 mL of vector (either Desmopan or silicone oil 20 cSt), 118 mL of MSM and ethanol at 1.0 g/l and 3.0 g/l. The bottles were magnetically agitated (300 rpm) for 4 days at 25°C, and aqueous samples were taken at days 3 and 4 to determine the residual aqueous ethanol concentration.

Oxygen transfer in reactors

An ALR and a STR were used as model reactor configurations. The ALR consisted of a glass column (0.090 m inner diameter, 0.415 m height, 2 L working volume) with a concentric tube (0.055 m inner diameter, 0.295 m height) placed at 0.04 m from the bottom of the reactor. The air flow rate was maintained at 1 vvm. The STR consisted of a glass reactor (0.1 m inner diameter, 0.145 m height, 1 L working volume) equipped with a double Rushton turbine and operated at 300 rpm. The air flow rate was maintained at 0.8 vvm. Both reactors were tested with 10% (*v*/*v*) of vector at 25°C. Abiotic experiments were carried out to determine the overall oxygen transfer rate (OTR) from the gas to the aqueous phase by periodically monitoring the time course of sulfite oxidation in a SRT and ALR, according to Quijano et al. (2009). This parameter describes the lumped oxygen transfer rate (gas/water + gas/vector/water pathways). The reactors were initially filled with a sodium sulfite solution (0.03 M) and the corresponding vector, and aerated for 30 min prior to experimentation. The kinetics of oxygen absorption were recorded after the addition of the catalyzer until complete sulfite depletion, according to Eq. 2.

$$SO_3^{2-} + 0.5O_2 \xrightarrow{Co^{2+}} SO_4^{2-} \quad (2)$$

Aqueous samples of 5 mL were taken from the bottom of the reactor and sulfite concentration was determined by iodometric back-titration according to Zhao et al. (1999). When liquid vectors were used, aeration and agitation were interrupted for 30 s in order to allow phase separation. After sampling, aeration and agitation were resumed. For a correct data analysis, time scales were corrected by discarding the time periods during which both aeration and agitation were stopped. A previous report confirmed that this method allowed the estimation of OTR with errors lower than 10% and that aeration and mixing interruptions do not affect overall mass transfer (Quijano et al. 2009).

Effect of vectors in *S. cerevisiae* cultures

The influence of the addition of vectors on *S. cerevisiae* metabolism was evaluated using the STR as model

bioreactor in glucose-supplemented cultures. The STR was initially filled with 720 mL of sterile MSM, 90 mL of the tested vector (either silicone oil 20 cSt or Desmopan), 90 mL of a 100 g L^{-1} glucose stock solution (in order to reach an initial glucose concentration of 10 g L^{-1}) and inoculated with *S. cerevisiae* at approximately 2 g dry weight L^{-1}. Control tests in the absence of a vector were also performed. Temperature, stirring, and aeration were maintained at 25°C, 300 rpm, and 0.25 vvm, respectively. Liquid samples of 5 mL were taken every 2 h to monitor pH, biomass, glucose, and ethanol concentrations over a 12-h experimentation period. When silicone oil was present, 1 mL samples of the aqueous organic dispersion were centrifuged for 10 min at 13,300 rpm and resuspended in 1 mL of fresh MSM prior to the determination of culture absorbance in order to avoid the interference of silicone oil. Additionally, both dissolved oxygen concentration and temperature were on-line measured with a polarographic oxygen probe connected to a DO transmitter (4100e, Mettler-Toledo, Germany). The cultures were carried out in duplicate.

Analytical procedures

Oxygen and CO_2 concentrations were determined in a gas chromatograph (Varian CP-3800, Palo Alto, CA, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m, 0.53 μm, 15 μm) and a CP-Pora BOND Q (25 m, 0.53 μm, 10 μm) columns. Oven, injector, and detector temperatures were maintained at 40, 150, and 175°C, respectively. Helium was used as the carrier gas at 13.7 mL min^{-1}.

Iodometric back-titration was performed with calibrated digital burettes (Bürette Digital III, Brand, Germany). Biomass production was evaluated by spectrophotometric measurements of culture absorbance at 600 nm (Hitachi U-2000 spectrophotometer). Absorbance at 600 nm was correlated to cell dry weight. Glucose and ethanol concentrations were analyzed via high-performance liquid chromatography-infrared spectroscopy (HPLC-IR) using a Waters 515 HPLC pump coupled with a refractive index detector (Water 410) and an Aminex HPX-87C column (Biorad). Samples were eluted isocratically at 85°C using water as the mobile phase at a flow rate of 0.6 mL min^{-1}.

Statistical analyses

Statistical analyses were performed with NCSS® software (Jerry Hintze 2001). Analysis of variance (α=0.05) and Tukey–Kramer tests were performed in order to find significant differences among the tests carried out. The error associated with the experimental procedures was expressed as the standard error of the mean.

Results

The selected liquid and solid vectors were first characterized in terms of toxicity, biodegradability, volatility, thermal stability (only solid polymers), and oxygen partitioning (Table 1). FC40 and Kraton exhibited the lowest values of $K_{g/v}$ (3.0 for both vectors), while Desmopan and Silicone oil 20 cSt presented the highest values (5.1 and 4.8, respectively). On the other hand, $K_{v/w}$ for ethanol in Desmopan and silicone oil 20 cSt were 0.12 and 0.35, respectively. Polymer thermal stability tests showed that Desmopan was not affected by the autoclaving process either with distilled water or with MSM. Minor melting and agglomeration was observed when Kraton was autoclaved with MSM, but surprisingly no significant effect was observed in the presence of distilled water. Complete melting of Elvax occurred during polymer autoclaving in both distilled water and MSM. The density of all the vectors tested ranged from 0.98 (silicone oil 20 cSt) to 1.12 g cm^{-3} (Desmopan). The kinematic viscosity of the liquid vectors ranged from 1.8 (FC40) to 200 cSt (silicone oil). None of the tested vectors was either biodegradable or toxic for *S. cerevisiae*. Significant differences in cost were observed, being the liquid vectors up to 250 times more expensive than the solid ones.

Volatilization tests showed that silicone oil was non-volatile, regardless of its viscosity. On the contrary, FC40 was rapidly volatilized, with 88% of the initial volume being evaporated after 30 days of aeration. Approximately 12% of the initial HMN had been volatilized by the end of the experimentation, however most of HMN volatilization occurred within the first 5 days.

Higher CO_2 production rates and oxygen consumption rates (initial slope of the curves up to three times higher than in the control) were observed in the presence of vectors during toxicity tests (Fig. 1). Based on the initial rates, silicone oil 20 cSt and Kraton supported the largest enhancements.

The actual oxygen transfer enhancement performance was determined via sulfite oxidation under abiotic operation in a SRT and ALR. In all cases, sulfite oxidation followed a zero order reaction (R^2>0.97). The enhancement factor, defined by Eq. 3, was used for comparative purposes,

$$E_f = \left(\frac{OTR_{TPPB}}{OTR_w} - 1\right) \times 100 \quad (3)$$

where E_f represents the mass transfer enhancement due to the presence of a vector, and OTR_{TPPB} and OTR_w the oxygen transfer rate in tests supplied with and without a vector, respectively. The presence of vectors enhanced the overall oxygen transfer, regardless of the bioreactor design. Desmopan was the vector with the highest E_f values (171%

Table 1 Properties and parameters evaluated during vector selection

Transfer vector	$K_{g/v}$ ($C_g \cdot C_L^{-1}$)	Thermal stability	Density (g cm^{-3})	Cost[a] (€ L^{-1})	ν[b] (cSt)	Size (mm)
Silicone oil 20 cSt	4.8±0.2	+	0.98	165	20	–
Silicone oil 200 cSt	3.6±0.2	+	0.98	232	200	–
HMN	3.4±0.1	+	0.79	764	4.2	–
FC40	3.0±0.2	+	1.85	276	1.8	–
Elvax	3.5±0.2	–	0.95	3	–	3–4 beads
Desmopan	5.1±0.2	+	1.12	7	–	3×3 cylinders
Kraton	3.0±0.3	−(MSM) + (H_2O)	0.90	4	–	3–4 beads

[a] Prices are given according to Sigma-Aldrich (silicone oils and HMN), 3 M (FC40), Dupont (Elvax), Kraton Polymers (Kraton), and Bayer (Desmopan) in 2009

[b] Kinematic viscosity

and 255% for the STR and ALR, respectively), while Kraton and Elvax supported the lowest mass transfer enhancements in both STR and ALR, with E_f ranging from 8% to 29% (Table 2). Higher E_f values were recorded with the liquid vectors in the STR (88%, 96%, and 89% for silicone oil 20 cSt, 200 cSt, and HMN, respectively) when compared with the ALR (73%, 44%, and 20% for silicone oil 20 cSt, 200 cSt, and HMN, respectively; Table 2). Finally, it is important to mention that all liquid vectors presented a reasonably good dispersion regardless of the bioreactor design (including FC40 that exhibited a density superior to water). All solid polymers preferentially remained at the top of the reactors (even Desmopan with a density of 1.12 g cm^{-3}) in both reactor designs, except for Elvax that showed good circulation in the ALR.

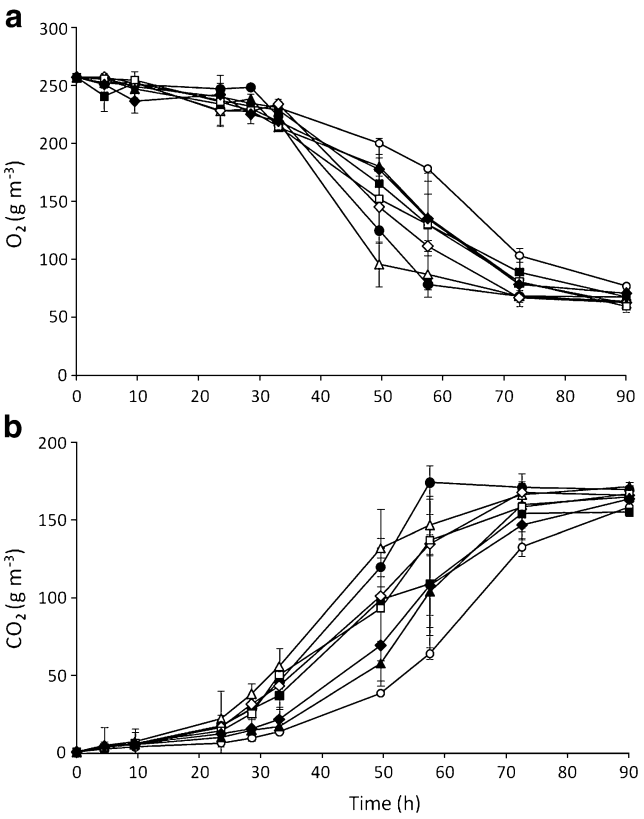

Fig. 1 Time course of oxygen consumption (**a**) and carbon dioxide production (**b**) during the degradation of glucose (10 g L^{-1}) by *S. cerevisiae* in tests supplied with Kraton (*filled circle*), Elvax (*filled triangle*), Desmopan (*unfilled square*), silicone oil 20 cSt (*unfilled triangle*), silicone oil 200 cSt (*unfilled diamond*), HMN (*filled square*), FC40 (*filled diamond*), and in the absence of vector (*unfilled circle*). *Vertical bars* represent the standard deviation from duplicates

Based on their biocompatibility, non-biodegradability, thermal stability, low cost, non-volatility, and high E_f values, silicone oil 20 cSt and Desmopan were selected for further investigation.

Experiments in the STR showed that in presence of silicone oil 20 cSt and Desmopan, *S. cerevisiae* concentration increased by approximately 50% and 70%, respectively, within the first 12 h of cultivation. In absence of vector, biomass concentration increased by only 25% during the same period of time (Fig. 2a). Biomass concentration steadily increased when Desmopan was employed, while *S. cerevisiae* growth only occurred within the first 4 and 6 h of cultivation in the control and silicone oil-supplemented tests, respectively. Glucose uptake rates were fastest in the fermentations carried out in the absence of a vector, while silicone oil 20 cSt supported the slowest glucose removal rates (Fig. 2b).

It must be stressed that the dissolved oxygen concentration always remained above 6.5 mg L^{-1} during most of the experimentation time with or without a vector (Fig. 2a). However, under these fully aerobic conditions ethanol production was observed. The rates of ethanol accumulation in the aqueous phase were significantly higher when the SRT was deprived of vector, with silicone oil supporting the lowest accumulation rates (1.2 g ethanol L^{-1} h^{-1} in the control vs. 0.7 g ethanol L^{-1} h^{-1} in fermentations supplied with silicone oil). Several authors have reported a combined oxidative/fermentative metabolism of *S. cerevisiae* at glucose concentrations above 0.15 g L^{-1} in batch cultures (Feria-Gervasio et

Table 2 Oxygen transfer rates and enhancement factors under abiotic conditions in a SRT and an ALR

	STR		ALR	
Transfer vector	OTR_{eff} (mgO_2 $L_{reactor}^{-1}h^{-1}$)	E_f (%)	OTR_{TPPB} (mgO_2 $L_{reactor}^{-1}h^{-1}$)	E_f (%)
No vector (control)	770±20	0	1,046±54	0
Silicone oil 20 cSt	1,445±28	88	1,811±176	73
Silicone oil 200 cSt	1,508±41	96	1,503±69	44
HMN	1,453±70	89	1,253±129	20
Elvax	997±10	29	1,131±11	8
Desmopan	2,083±57	171	3,710±156	255
Kraton	946±36	23	1,252±60	20

al. 2008; Meijer et al. 1998; Otterstedt et al. 2004). Thus, the ethanol production was an additional indicator of the effect of vectors on the metabolism of *S. cerevisiae*.

Discussion

None of the vectors tested was either biodegradable or inhibited glucose oxidation by *S. cerevisiae* (a key selection criterion in the mass cultivation of the yeast), which made them suitable for use in TPPBs-based fermentations. Liquid vector volatility, despite being an obvious criterion for vector selection, has been traditionally taken for granted, probably due to the non-recommended practice of basing solvent selection on previous reported works on the topic (Amaral et al. 2008; Cesário et al. 1998; Elibol 2001). In addition, it is difficult to predict the volatility of several liquid vectors since physical properties such as vapor pressures are scarce in the literature. The perfluorocarbon FC40, which is among the most commonly studied vectors for enhancing oxygen transfer in fermentation processes (Amaral et al. 2006, 2008; Elibol and Mavituna 1999; Pilarek and Szewczyk 2008), was extremely volatile (88% after 30 days). In addition to the high costs resulting from FC40 losses (276 € L^{-1}), this vector has been classified as an ozone depleting substance (Toft et al. 2006). From these results, FC40 was therefore discarded as an oxygen transfer vector. The HMN volatilization pattern (12% of the initial solvent volatilized after 30 days with 8% in the first 6 days) suggested that only an unidentified fraction of the vector was lost, which is surprising as HMN is supposedly a pure compound. Silicone oils were highly stable over the experimentation period regardless of their viscosity. This is not surprising as vapor pressures as low as 10×10^{-14} atm have been reported for silicone oils of 705 cSt (Chen et al. 2006).

Vector thermal stability is also critical in TPPBs-based fermentations where culture broth sterilization is required. Among the solid vectors tested, Desmopan was the only polymer exhibiting thermal stability. In the case of Kraton, the thermal stability was surprisingly affected by the type of matrix (either mineral salt medium or distilled water).

The $K_{g/v}$ herein obtained for oxygen ranged from 3.0 to 5.1 and were comparable to those previously reported in literature (5.7 and 2.1 for silicone oil and perfluorocarbon, respectively; Muñoz et al. 2007). In our study, the maximum enhancements in the overall oxygen transfer rate corresponded surprisingly to the vectors exhibiting the highest values of $K_{g/v}$ (silicone oil 20 cSt and Desmopan). Different E_f were found for each vector tested in the two reactor designs and different E_f were recorded within each reactor for the different vectors, which indicates there is an

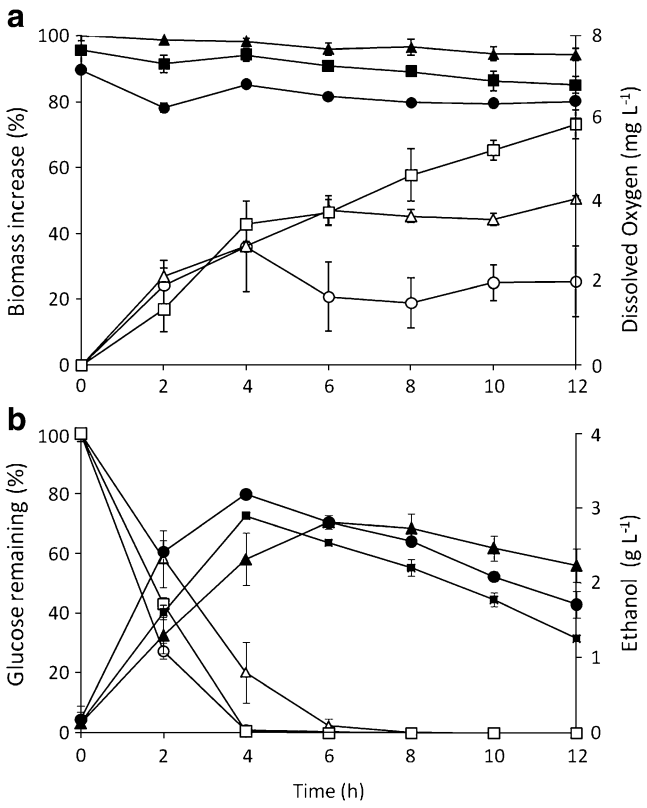

Fig. 2 Time course of **a** biomass increase and dissolved oxygen concentration and **b** glucose remaining and ethanol production in *S. cerevisiae* fermentations supplied with glucose (10 g L^{-1}) in the absence of vector (*circles*), in the presence of silicone oil 20 cSt (*triangles*), or in the presence of Desmopan (*squares*). *Open symbols* refer to the main *Y* axis while *closed symbols* refer to the secondary *Y* axis

optimum vector–reactor pair. In this context, the equilibrium concentration (C^*_{mix}) of the gaseous substrate in the overall liquid (water + vector) can be defined as:

$$C^*_{mix} = f_w \frac{C_g}{K_{g/w}} + f_v \frac{C_g}{K_{g/v}} \quad (4)$$

where f_w and f_v represent the volumetric fractions of water and vector, respectively, and $K_{g/w}$ represents the gas/water partitioning coefficient. Under the experimental conditions evaluated in this study, the value of C^*_{mix} for Kraton (the polymer with the highest affinity for oxygen, $K_{g/v}$=3) was 16.4. This represents an increase of 220% in the concentration gradient available for mass transfer compared to same system without vector. However, only a 20% increase on E_f was observed with Kraton in both reactors. On the contrary, from Eq. 4 the value of C^*_{mix} for Desmopan (the vector with the lowest affinity for oxygen, $K_{g/v}$=5), was 12.8. This represents an increase of 175% in the concentration gradient compared to the same system without vector. However, a 255% of increase on E_f was observed with Desmopan in the ALR. These results clearly show that the oxygen affinity for the vector (quantified through $K_{g/v}$) did not correlate with E_f. For this reason, the simple model of averaged solubilities is not always a good theoretical framework to understand the effect of vectors on mass transfer.

These results also suggest that the presence of a vector not only influenced the concentration gradient available for transfer, but also physical enhancements on K_La should be considered. Zhang et al. (2006) proposed that the continuous collisions of either oil droplets or solid particles can increase the turbulence in the gas/liquid interface. Moreover, the gas-bubble disruption capacity of the vectors observed previously by Galindo et al. (2000) could account for a higher value of the gas/liquid and gas/solid interfacial area. Hence, a larger effect of the vector on K_La than on the concentration gradient would explain the apparent mismatch between higher mass transfer enhancements by the vectors with the lowest affinity for oxygen. In this context, both silicone oil and Desmopan exhibited a very favorable hydrodynamic behavior (good dispersion) during STR operation. In the ALR, Desmopan remained entrapped in the upper section of the draft tube acting as an efficient air bubble disruptor, which likely increased the gas/liquid interfacial area for mass transfer ($E_f \approx 255\%$ compared with 8% and 20% in the case of Elvax and Kraton, respectively). These results agree with those previously reported by Olle et al. (2006) who observed that particles of magnetite (Fe_3O_4) significantly increased the gas/liquid interfacial area, this effect being even more important than an increased turbulence in the gas/liquid interface. Likewise, Littlejohns and Daugulis (2007) observed a mechanically mediated enhancement of oxygen transfer to the aqueous phase in stirred tank reactors supplied with glass beads (volumetric mass transfer coefficients up to 120% higher than in single air-aqueous dispersions), this enhancement being larger than with solid polymers exhibiting higher affinities for oxygen (i.e., silicone beads). Therefore, the variables influencing K_La such as changes in the surface tension, gas bubbles disruption, and vector hydrodynamics established within the reactor determine the overall oxygen transfer and thus E_f should replace $K_{g/v}$ as a selection criterion.

The CO_2 production and oxygen consumption patterns recorded in the toxicity tests suggested a higher glucose oxidation in the presence of vectors (Fig. 1a, b). This might be due to a vector-enhanced supply of oxygen to *S. cerevisiae*, which triggered aerobic yeast metabolism and thus reduced the production of ethanol. In the SRT, the presence of a vector, either silicone oil 20 cSt or Desmopan, also affected *S. cerevisiae* metabolism, as shown by the differences in biomass production, glucose uptake, and ethanol accumulation (Fig. 2). The addition of Desmopan increased biomass production by approximately 300% compared to the fermentations carried out in the absence of a vector, which is in accordance with the results of Pilarek and Szewczyk (2008) who reported an increase in *S. cerevisiae* growth of 170% in the presence of 6% of perfluorodecalin (a perfluorocarbon-based vector) at low agitation rates (100 rpm). Likewise, Jia et al. (1996) recorded a *S. cerevisiae* increase of 100% using 4% of a mixture of alkanes in a tower bioreactor. Despite the fact that the use of vectors to enhance the growth of *S. cerevisiae* has been extensively studied, the mechanisms involved in this enhancement are usually attributed exclusively to an enhanced transfer of oxygen to the cultivation medium. To date, not enough studies have been carried out assessing the key parameters involved in *S. cerevisiae* metabolism: biomass, glucose, dissolved oxygen, and ethanol concentrations.

The oxygen transfer enhancements obtained for silicone oil and Desmopan under abiotic conditions in the SRT (88% and 171%, respectively) did not result in comparable enhancements in biomass growth and glucose uptake rates. It is worth noting that dissolved oxygen concentrations higher than 6 mg L^{-1} were always recorded regardless of the experimental conditions evaluated. These high oxygen concentrations were unexpected based on both the high biomass concentrations present in the bioreactor and the low aeration rates provided. Moreover, lower glucose uptake rates were recorded in the presence of a vector. These differences might be explained by the fact that at the glucose concentrations employed in this study (10 g L^{-1}), *S. cerevisiae* metabolism was limited by a glucose-induced catabolic repression, rather than by the dissolved oxygen

concentration in the cultivation medium as previously reported by Feria-Gervasio et al. (2008). This glucose-mediated repression, reported in *S. cerevisiae* cultures at high glucose concentrations, thus resulted in a simultaneous oxidative and fermentative metabolism, with the subsequent release of ethanol to the cultivation medium (Fig. 2).

Otterstedt et al. (2004) reported that, under oxidative metabolism, the specific glucose uptake rate of *S. cerevisiae* is lower than under fermentative conditions. This decrease in the glucose uptake rate was clearly observed in the presence of both silicone oil 20 cSt and Desmopan. Moreover, the lower ethanol production and the higher biomass yields confirmed that the vectors significantly stimulated the oxidative metabolism of *S. cerevisiae*. Since no oxygen limiting conditions were observed in the cultures without vectors, this effect on the metabolism cannot be explained in terms of mass transfer enhancement, as previously assumed (Jia et al. 1996; Pilarek and Szewczyk 2008). Moreover, the low affinity of the vectors for ethanol suggests that a toxicity reduction by ethanol partitioning into the vectors was not involved in the enhancement recorded. No clear and robust explanation for these empirical findings can be given with certainty based on the high complexity of *S. cerevisiae* metabolism. In this context, MacLeod and Daugulis (2005) also observed a decreased glucose uptake rate by *Mycobacterium* PYR-1 in the presence of the vectors HMN and bis(ethylhexyl) sebacate. These authors proposed that this decrease was due to the high affinity of the cells towards the vectors. Therefore, the effect of the vectors on cell metabolism can be due to complex cell-vector interactions and further research must be carried out to understand this phenomenon.

The results obtained herein clearly show that the oxygen affinity for the vector (quantified through $K_{g/v}$) did not correlate with E_f. Therefore, a simple model of averaged solubilities is not always a good theoretical framework to understand the effect of vectors on mass transfer. Moreover, our results suggest that the vectors improved the oxygen transfer by means of physical enhancements on K_La rather than by a concentration gradient enhancement. Then, variables influencing K_La such as changes in the surface tension, viscosity, bubble disruption, and vector hydrodynamics determine the overall oxygen transfer and thus E_f should replace $K_{g/v}$ as a selection criterion. In *S. cerevisiae* fermentations, a clear stimulation of the oxidative metabolism was observed in the presence of vectors (higher biomass production, lower ethanol excretion, and lower glucose uptake rate). Surprisingly, this effect on the *S. cerevisiae* metabolism was not related to OTR improvements. No clear explanation for this empirical finding can be given but the stimulation of the oxidative metabolism might be related to complex cell–vector interactions and further research must be done. Based on its lower improvement on biomass growth and the operation problems derived from its use (intensive foaming, vector adherence to the bioreactor wall), the use of silicone oil is seriously questioned. The low cost of solid polymers such as Desmopan together with the observed prevention of culture foaming (mechanical foam disruption), the easy vector recovery and recycling, and the sustained biomass production supported, make Desmopan a promising candidate to improve the performance of industrial fermentations.

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